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Molecular Genetic Screening in Cytogenetically Normal Mentally Retarded Males With Manifestations of Fragile X Syndrome

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To the Editor

Due to phenotype variability, early recognition of fragile X (fra(X)) individuals can be difficult. Butler et al. [1991a, b] and Hagerman et al. [1991] reported that certain physical characteristics (e.g., ear width, testicular volume, bizygomatic diameter, head breadth, plantar crease, hyperflexibility) could be used to correctly classify the fra(X) syndrome in over 90% of mentally retarded males. In addition, an anthropometric survey using multivariate analysis was reported on 110 mentally retarded males (39 with and 71 without the fra(X) syndrome) and significant differences were found for 18 measurements (weight, height, 7 linear, 2 breadth, 5 craniofacial, 1 skinfold, and testicular volume) with the greatest measurements (excluding bizygomatic diameter) found in fra(X) males [Butler et al., 1991a]. Discriminant analysis resulted in a discriminant function based on four of 30 anthropometric variables and age. The four variables in the discriminant function were the Z scores representing testicular volume, ear width, bizygomatic diameter, and head breadth. Patients with the fra(X) syndrome were distinguished from mentally retarded males without the fra(X) syndrome at an overall correct classification rate of 95%.

Butler et al. [1991b] also reported the use of a 15-item checklist of physical and historical variables in screening for the fra(X) syndrome in 188 mentally retarded males (19 with and 169 without the fra(X) syndrome). Significant differences were found between mentally retarded males with and without the fra(X) syndrome with increased hyperactivity; shorter attention span; greater tactile defensiveness, hand-flapping, perseverative speech, and hyperextensibility; larger ears and testes; higher frequency of simian creases or Sydney lines and plantar creases; and more frequently a family history of mental retardation in the fra(X) syndrome males. A discriminant analysis of the 15 checklist variables and age in the 188 males resulted in a discriminant function based on 6 variables (plantar crease, simian crease, hyperflexibility, large testes, large ears, and a family history of mental retardation). Males with the fra(X) syndrome were distinguished from mentally retarded males without the fra(X) syndrome at an overall correct classification rate of 93%.

In order to identify if cytogenetically normal (using two induction systems [folate deficient culture medium and folate replete culture medium but with 5-fluorodeoxyuridine] to

enhance the expression of fragile sites) yet mentally retarded males without a known cause of their retardation had expansion of the CGG repeat segment of the FMR-1 gene, Southern blot analysis was performed using two probes (fxa241 [Oncor, Inc., Gaithersburg, MD] and Ox1.9 [kindly provided by Dr. Kay Davies]) following digestion of genomic DNA with PstI and EcoRI/EagI, respectively, using established protocols [Kremer et al., 1991; Rousseau et al., 1991]. DNA studies were performed on 20 (12.3%) of the previously examined 162 (122 whites and 40 blacks; age range 7–77 years with an average age of 40.0 ± 14.4) cytogenetically normal mentally retarded males without a known cause of their retardation but with high anthropometric discriminant values and/or clinical checklist scores as described elsewhere [Butler et al., 1991a, b]. The average discriminant value $\pm$ SD was 1.9 ± 1.5 with a range of -0.5 – 4.9 and average checklist score $\pm$ SD was 19.0 ± 5.4 with a range of 6–26 for 21 fra(X) syndrome males previously studied, while the average discriminant value $\pm$ SD was -0.5 ± 1.0 with a range of -3.0 – 3.1 and average checklist score $\pm$ SD was 8.0 ± 4.0 with range of 2–22 for the 162 cytogenetically normal mentally retarded males utilized in this study. None of the 20 cytogenetically normal mentally retarded males with physical and behavioral manifestations of the fra(X) syndrome (average discriminant value $\pm$ SD was 0.9 ± 0.6 with a range of 0–2.0 and average checklist score $\pm$ SD was 11.4 ± 3.9 with a range of 2–18) showed expansion of the CGG repeat of the FMR-1 gene detectable with the two probes used in this study.

Our study further supports that major FMR-1 gene amplification is extremely rare in cytogenetically normal mentally retarded males with physical and behavioral changes of the fra(X) syndrome. While heterogeneous single base pair substitutions, small deletions, or insertions in the FMR-1 gene could produce a deficiency in the FMR-1 protein (FMRP) [Wöhrle et al., 1992; De Boulle et al., 1993; de Vries et al., 1993; Ashley et al., 1993], aberrations in other X-linked mental retardation genes, not identified to date, could also play a role in the causation of mental retardation in cytogenetically normal males.

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